Figure 1 Anti-CD3 WT

GG GGGTCTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTC TGACATCTGAGGACTCTGCAGTCTATTACTGCAAGATATTATGATGATCATTACTGCCTTGA AGATGGATTATGACACATCCAAAGTGGCTTCT GGAGTCCCTTATCGCTTCAGTGGCAGTCTGGGACCTCATACTCTCTCACAATCAGCAGCA CATCTCCAGGGGGAGAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACTG 'AGC(GGAAGTGGAGGTTGGAGTCGACGACATTCAGCTGACCAGTCTCAGTATCATGTCT GATGCACTGGGTAAAACAGAGGCCTGGAC TCCTCAGTCGAAGGTGGAAGTTCGGGTTCTGG GATATCAAACTGCAGTCAGGGGGTTGAACTGGCAAGACCTGGGGGCCTCAGTGAAGATGTC TGGAGGCTGAAGATGCCGCCACTTACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTC AAGGACAAGGCCACATTGACTACAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGC GCAAGACTTCTGGCTACACCTTTACTAGGTACAC CTACTGGGCCAAGGCACCACTCTCACAGTC GTACCAGCAGAAGTCAGGCACCTCCCCAAA TGCTGGGACCAAGCTGGAAA

AA Sequence

SGGSG VTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVAS DIKLOOSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKF KDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGG GVPYRESGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK GSGGSGGVDDIQLTQSPAIMSASPGEK

Fig. 2 A

TH 2

GYTNYAQKLQGRVTMTTDTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWG SGYTATRYTMHWVRQAPGOGLEWIGYINPSR DVQLVQSGAEVKKPGASVKVSCKA QGTTVTVSS

VH3

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSR **GYTNYAQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDDHYCLDYWG** QGTTVTVSS

VH5

GYTNYADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWG DVOLVOSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR OGTTVTVSS

VH7

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR YMELSSLRSEDTAVYYCARYYDDHYCLDYWG GYTNYNQKFKDRVTITTDKSTSTA QGTTVTVSS

Fig. 2 A (cont.)

5

SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG DIQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPGKAPKRWIYDT TKVEIK

いら

SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG DIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPGKAPKRWIYDT TKVEIK

VL3

INSLEAEDAATYYCQQWSSNPLTFGGG DIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPGKAPKRWI SKVASGVPARFSGSGSGTDYSLT TKVEIK

Fig. 2B

CCGTGGTTATACTAATTACGCACAGAAGTTGCAGGG ATATTATGATGATCATTACTGCCTTGACTACTGGGG CTGGACAGGG CAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTC CCTGGGGCCTCAGTGAAGGTGTCCT CTGGGTAAGGCAGGCAC AGTGAAAAAA GCA CACGAT AAGGCTTCTGGCTACACCGCTACTAGGTA GACGTCCAACTGGTGCAGTCAGGGGCTGA CTGGAATGGATTGGATACATTAATCCTAĞ CGCGTCACAATGACTACAGACACTTCCAC GAGGACACTGCAACCTATTACTGTGCAAG CAAGGCACCACGGTCACCGTCTCA

7H3

ATATTATGATGATCATTACTGCCTTGACTACTGGGGC CAGCACAGCCTACCTGCAAATGAACAGCCTGAAAACT CCGTGGTTATACTAATTACGCACAGAAGTTGCAGGGC AAGGCTTCTGGCTACCGCTACTAGGTACACGATGCACTGGGTAAGGCCAGGCACCTGGACAGGG CCTGGGGCCTCAGTGAAGGTGTC AGTGAAAAA GACGTCCAACTGGTGCAGTCAGGGGCTGA CTGGAATGGATTGGATACATTAATCCTAG CGCGTCACAATGACTACAGACACTTCCAC GAGGACACTGCAGTCTATTACTGTGCAAG CAAGGCACCACGGTCACCGTCTCA

VHS

CCGTGGTTATACTACGCAGACAGCGTCAAGGGC CAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT CACGATGCACTGGGTAAGGCAGGCACCTGGACAGGG ATATTATGATGATCATTACTGCCTTGACTACTGGGG AGTGAAAAACCTGGGGCCTCAGTGAAGGTGTCCT GACGTCCAACTGGTGCAGTCAGGGGCTGA AAGGCTTCTGGCTACACCTTTACTAGGTA CTGGAATGGATTGGATACATTAATCCTAG CGCTTCACAATCACTACAGACAAATCCAC GAGGACACTGCAACCTATTACTGTGCAAG CAAGGCACCACGGTCACCGTCTCA

Fig. 2 B (cont.)

GAGGACACTGCAGTCTATTACTGTGCAAGATATTATGATGATTATTACTGCCTTGACTACTGGGGC CACTGGGTAAGGCAGGCACCTGGACAGGGT CIGCGIICI AAGGAC AGTGAAAAACCTGGGGCCTCAGTGAAGGTGTCCT CCGTGGTTATACTAATTACAATCAGAAGTTC CAGCACAGCCTACATGGAACTGAGCAGC AAGGCTTCTGGCTACACCTTTACTAGGTA CTGGAATGGATTGGATACATTAATCCTAG CGCGTCACAATCACTACAGACAAATCCAC GACGTCCAACTGGTGCAGTCAGGGGCTGA CAAGGCACCACGGTCACCGTCTCA

Fig. 2 B (cont.)

AGATGGATTTATGACACATCCAAGTGGCTTCTGGAGTCCCTGCTTCGCTTGGCAGTGGGTCT CTGTCTGCATCTGTCGGGGACCGTGTCACCATCACC TGCAGAGCCAGTCAAAGTTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCCAAAA GGGACCGACTACTCTCACAATCAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGGACCAAGGTGGAGATCAAA GACATTCAGATGACCCAGTCTCCATCTAG

VI 2

TGCAGAGCCAGTCAAAGTTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCAAAA AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTTCAGTGGCAGTGGGTCT GGGACCGACTACTCTCACAATCAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

VI3

AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTTCGCTGGCAGTGGGTCT TGCAGAGCCAGTTCAAGTTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCAAAA GGGACCGACTACTCTCACAATCAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTCTCCAGGGGAGCGTGCCACCTGA TGGCGGGACCAAGGTGGAGATCAAA CAGTGGAGTAGTAACCCGCTCACGTTCGG Fig. 2 C

vH CDR1

Wt anti-CD3

VH2,3

VH5,7

GYTFTRYTMH

GYTATRYTMH

GYTFTRYTMH

vH CDR2

WT anti-CD3,

VH7

VH5

VH2, 3

YINPSRGYTNYNQKFKD

YINPSRGYTNYADSVKG

YINPSRGYTNYAQKLQG

vH CDR3

WT anti-CD3,

VH2, 3, 5, 7

YYDDHYCLDY

vK CDR1

WT anti-CD3,

VL3

VL1, 2

RASSSVSYMN

RASQSVSYMN

vK CDR2

WT anti-CD3,

VL1, 2, 3

DTSKVAS

vK CDR3

WT anti-CD3,

VL1, 2, 3

QQWSSNPLT

Fig. 2 D

vH CDR1

WT anti-CD3 GGCTACACCTTTACTAGGTACACGATG

CAC

VH2,3 GGCTACACCGCTACTAGGTACACGATG

CAC

VH5,7 GGCTACACCTTTACTAGGTACACGATG

CAC

vH CDR2

WT anti-CD3,

VH7 TACATTAATCCTAGCCGTGGTTATACT

AATTACAATCAGAAGTTCAAGGAC

VH5 TACATTAATCCTAGCCGTGGTTATACT

AATTACGCAGACAGCGTCAAGGGC

VH2,3 TACATTAATCCTAGCCGTGGTTATACT

AATTACGCACAGAAGTTGCAGGGC

VH CDR3

WT anti-CD3,

VH2, 3,

VH5, 7 TATTATGATGATCATTACTGCCTT

GACTAC

Fig. 2 D (cont.)

vK CDR1

WT anti-CD3,

VL3 AGAGCCAGTTCAAGTGTAAGTTACATG

AAC

VL1, 2 AGAGCCAGTCAAAGTGTAAGTTACATG

AAC

vK CDR2

WT anti-CD3,

VL1-3 ACACATCCAAAGTGGCTTCT

VK CDR3

WT anti-CD3,

VL1-3 CAACAGTGGAGTAGTAACCCGCTCACG

A) anti-CD3 (VH2/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH2/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAP GQGLEWIGYINPSRGYTNYAQKLQGRVTMTTDTSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

C) anti-CD3 (VH2/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGT-CAAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-CAAGGTGGAGATCAAA

D) anti-CD3 (VH2/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

E) anti-CD3 (VH2/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-` CAAGGTGGAGATCAAA

F) anti-CD3 (VH2/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 4

A) anti-CD3 (VH3/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAA ATGAACAGCCTGAAAACTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH3/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDD-HYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 4 C) anti-CD3 (VH3/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA CAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAA ATGAACAGCCTGAAAACTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH3/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAP GQGLEWIGYINPSRGYTNYAQKLQGRVTMTTDTSTSTAYLQ MNSLKTEDTAVYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

E) anti-CD3 (VH3/VL3)

GACGTCCAACTGGTGCAGTCAGGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-CACTTCCACCAGCACAGCCTACCTGCAAATGAACAGCCT-GAAAACTGAGGACACTGCAGTCTATTACTGTGCAAGATATT ATGATGATCATTACTGCCTTGACTACTGGGGCCCAAGGCAC-CACGGTCACCGTCTCCTCAGGCGAAGGTACTAG-TACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGAC-GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTC TCCAGGGGAGCGTGCCACCCTGACCTGCAGAGCCAGTT-CAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGG-CAAGGCACCCAAAAGATGGATTTATGACACATCCA-AAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGG TCTGGGACCGACTACTCTCTCACAATCAACAGCTTG-GAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTG-GAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTG-GAGATCAAA

F) anti-CD3 (VH3/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-AQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 5 A) CD3 (VH5/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCAGACAGCGTCAAGGGCCGCTTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) CD3 (VH5/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAP GQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGGADDIQMTQSPSSLSASVGDRVTITCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

C) anti-CD3 (VH5/VL2)

GACGTCCAACTGGTGCAGTCAGGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACGCAGACAGCGTCAAGGGCCGCTTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH5/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAP GQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYME LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT STGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQ SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

Figure 5

E) anti-CD3 (VH5/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCTTTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACG-CAGACAGCGTCAAGGGCCGCTTCACAATCACTACAGACA-AATCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAG-CAGACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCT CTGTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGC-CAACAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGG-GACCAAGGTGGAGATCAAA

F) anti-CD3 (VH5/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYTNY-ADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 6

A) anti-CD3 (VH7/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACAATCAGAAGTTCAAGGACCGCGTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH7/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-

NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD
IQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

C) anti-CD3 (VH7/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG TGGTTATACTAATTACAATCAGAAGTTCAAGGACCGCGTCA CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA CTGAGCAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTG TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC GACTACTCTCACAATCAACAGCTTGGAGGCTGAAGATGC TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH7/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 6

E) anti-CD3 (VH7/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-AACCTGGGGCCTCAGTGAAGGTGTCCTG-CAAGGCTTCTGGCTACACCTTTACTAGGTACACGATG-CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT TGGATACATTAATCCTAGCCGTGGTTATACTAATTACAAT-CAGAAGTTCAAGGACCGCGTCACAATCACTACAGACA-AATCCACCAGCACAGCCTACATGGAACTGAG-CAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTGTGCAA GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT GTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGAC-CAAGGTGGAGATCAAA

F) anti-CD3 (VH7/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-QAPGQGLEWIGYINPSRGYT-NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-DAATYYCQQWSSNPLTFGGGTKVEIK

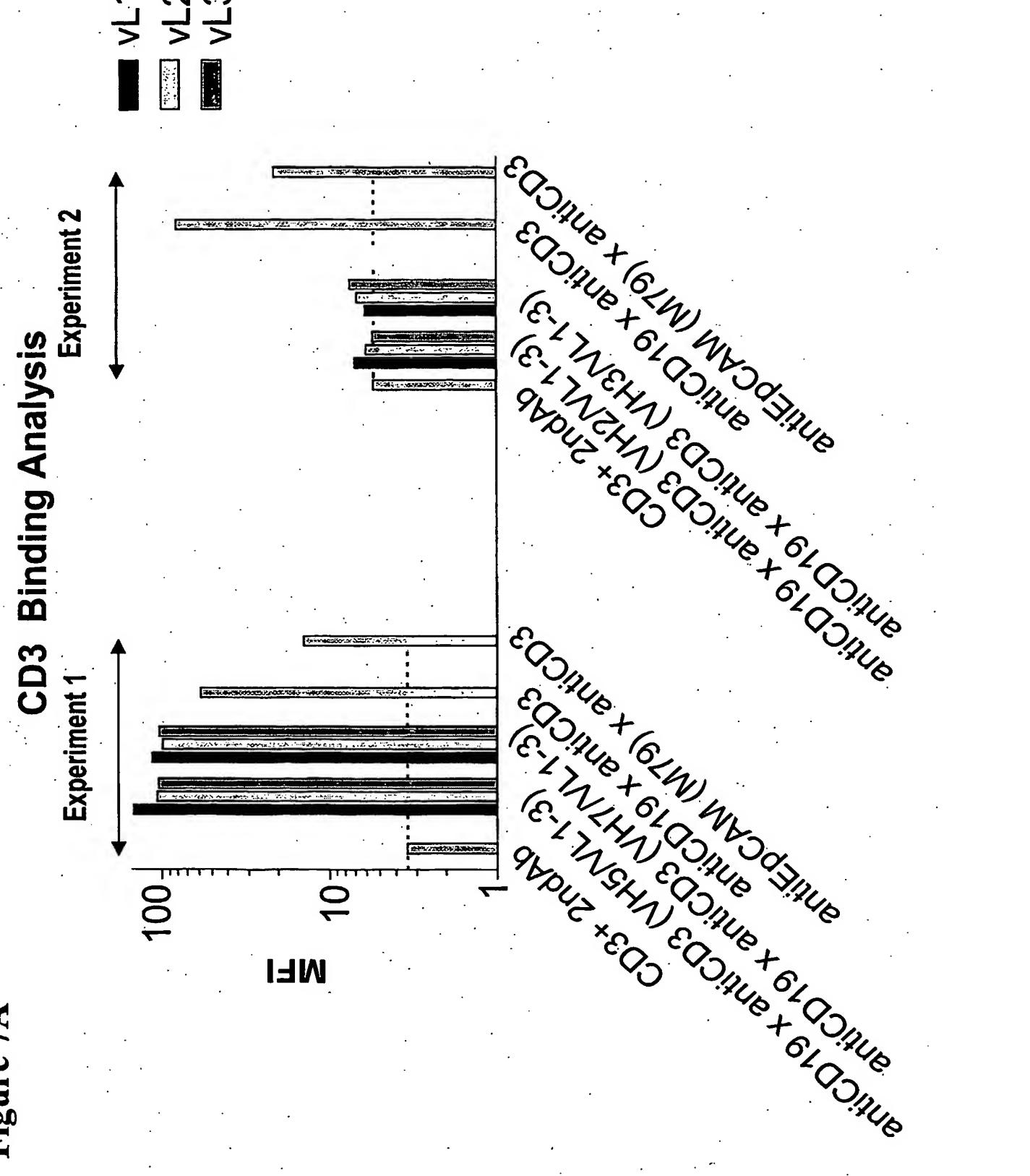
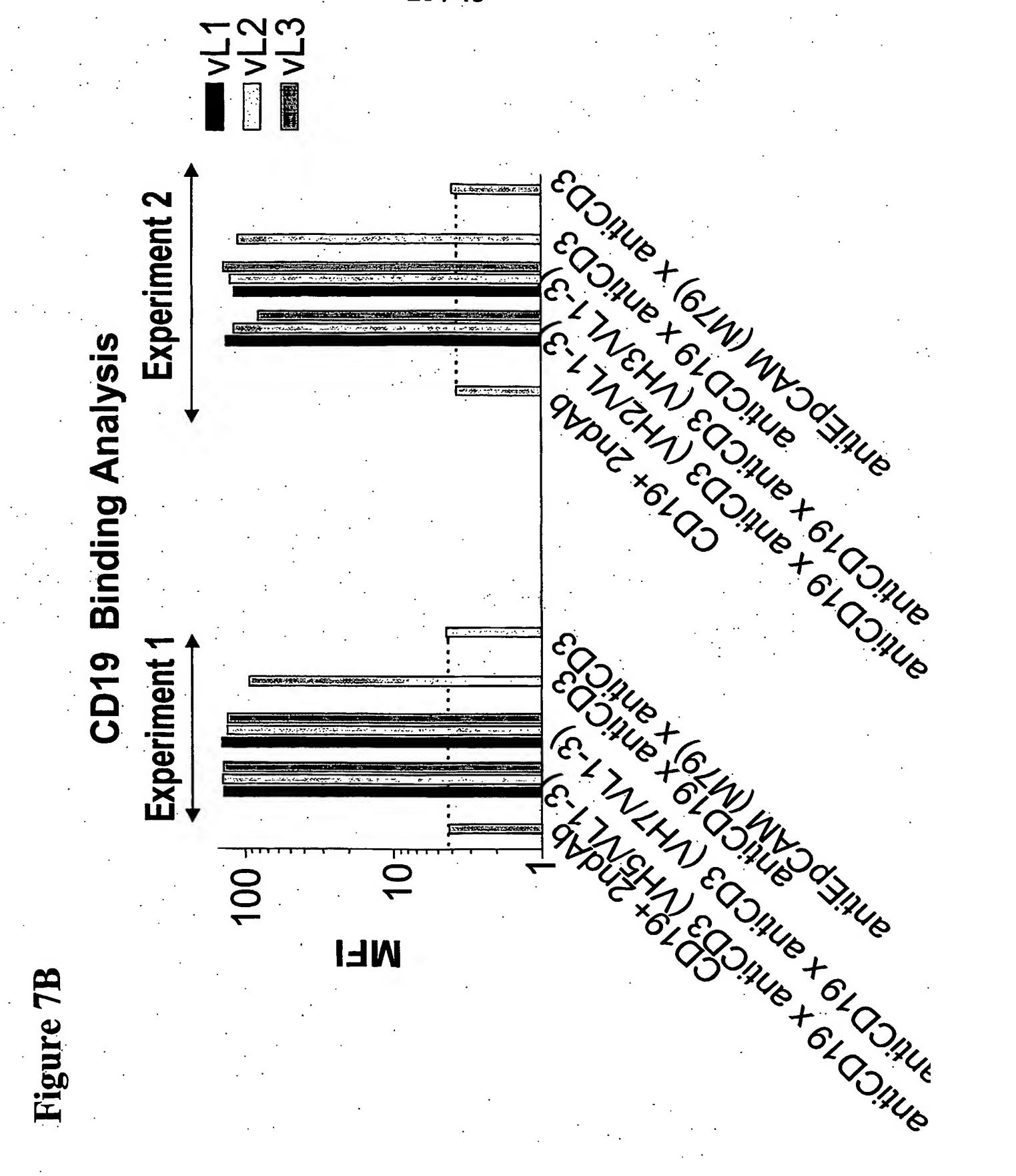


Figure 7.



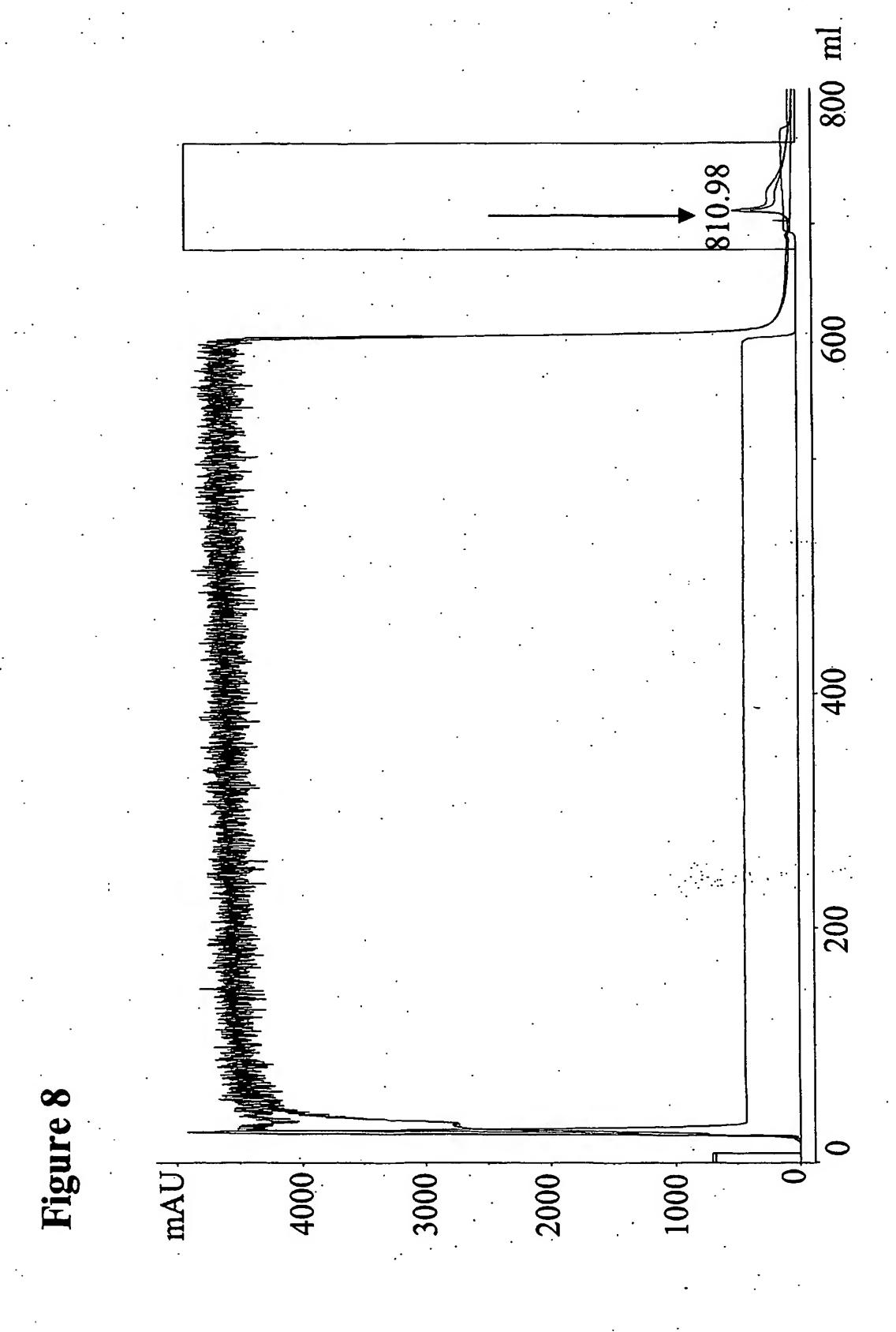


Figure 9

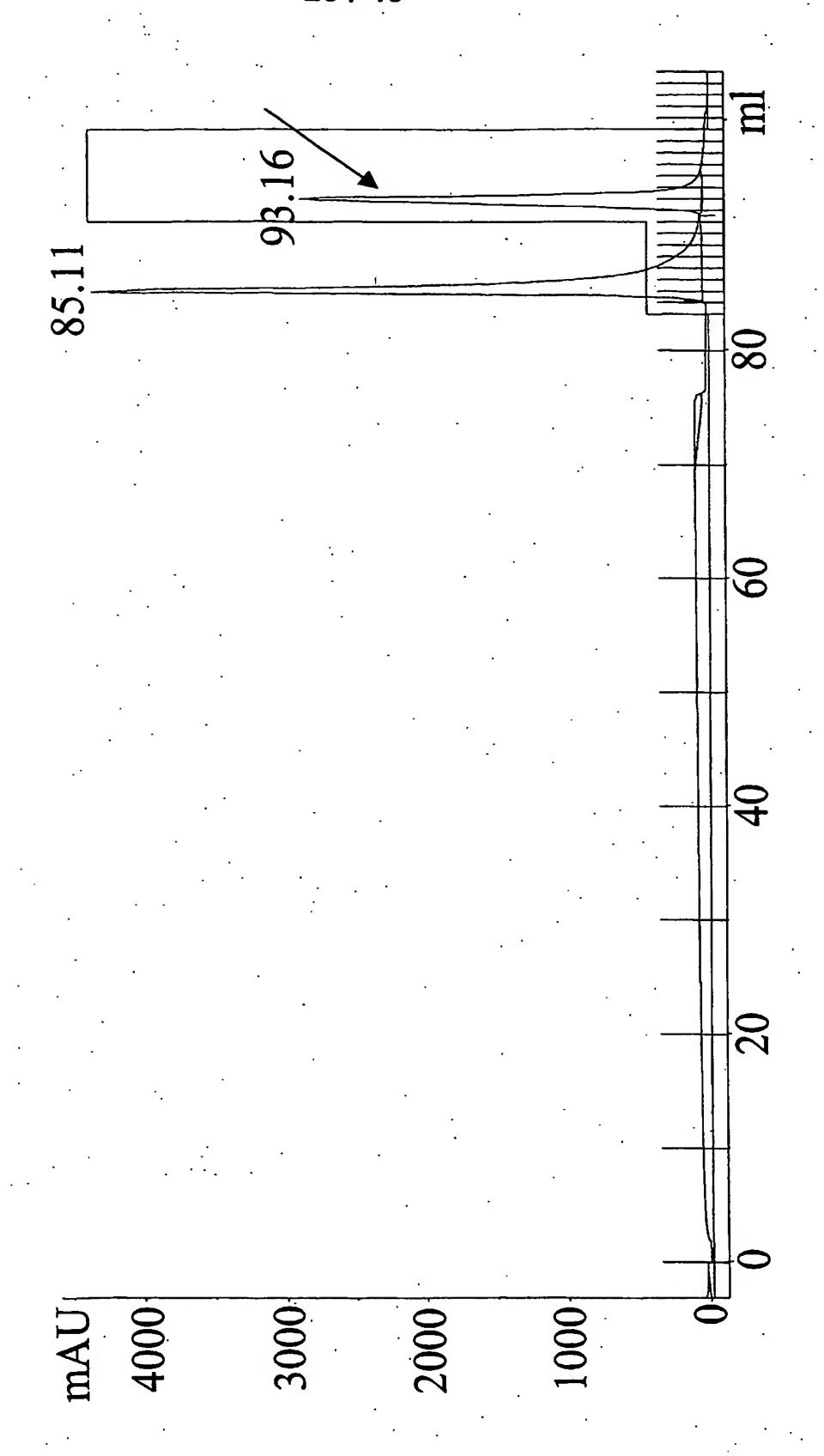


Figure 10

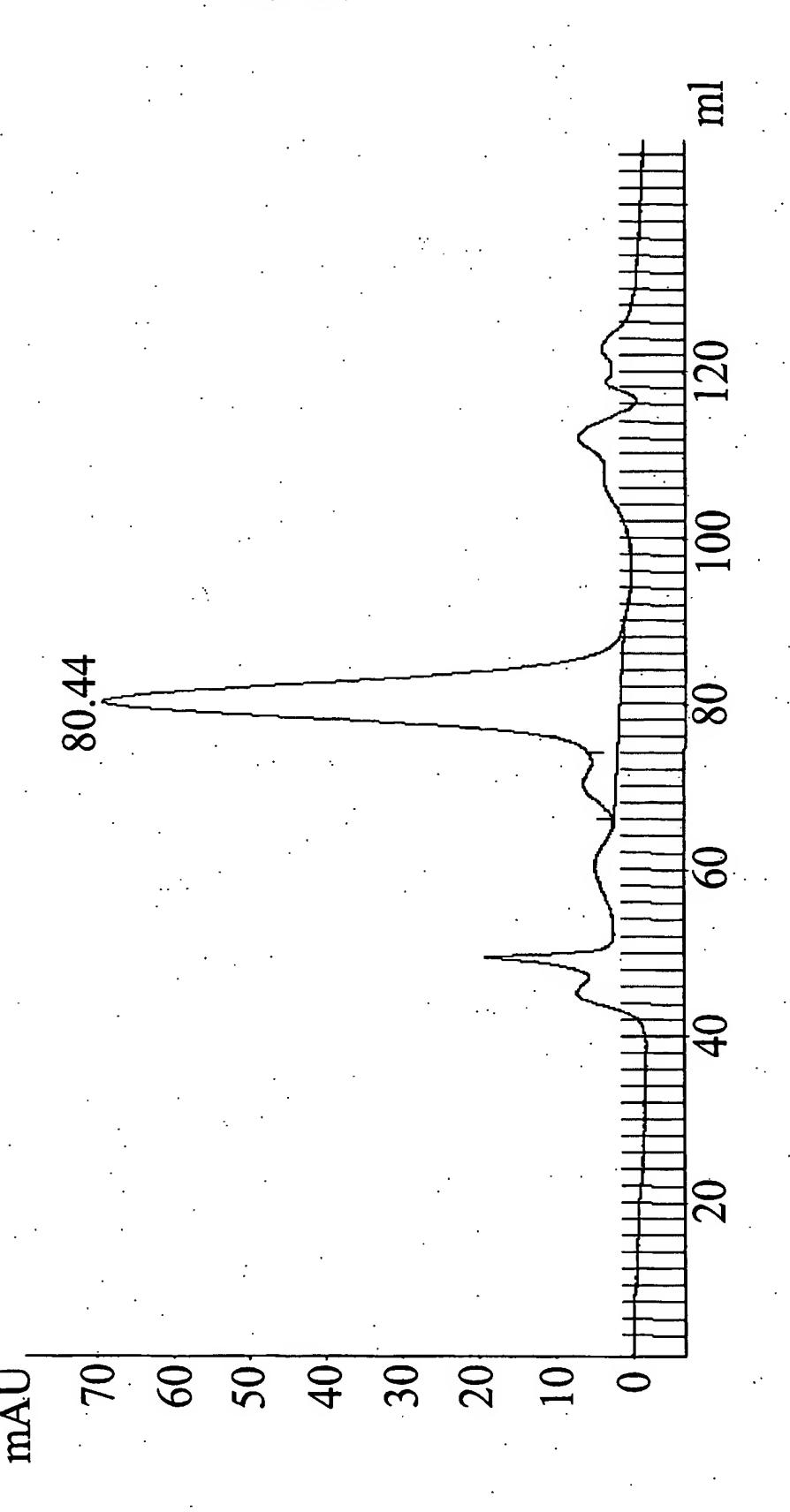


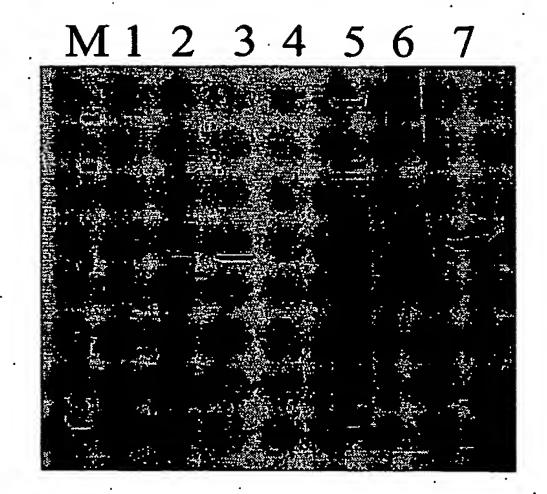
Figure 11

A)

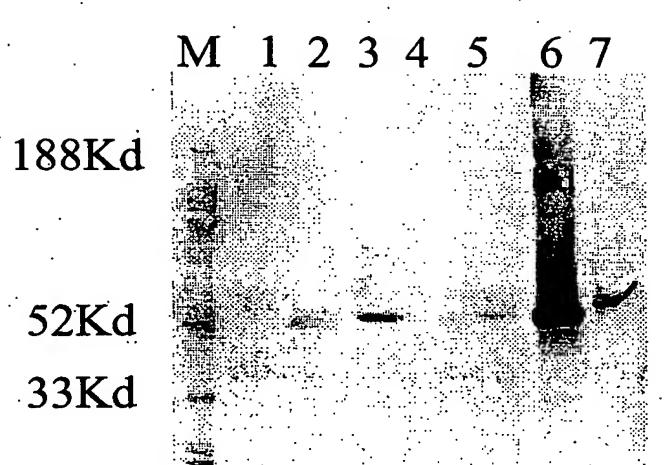
188KD

52 Kd

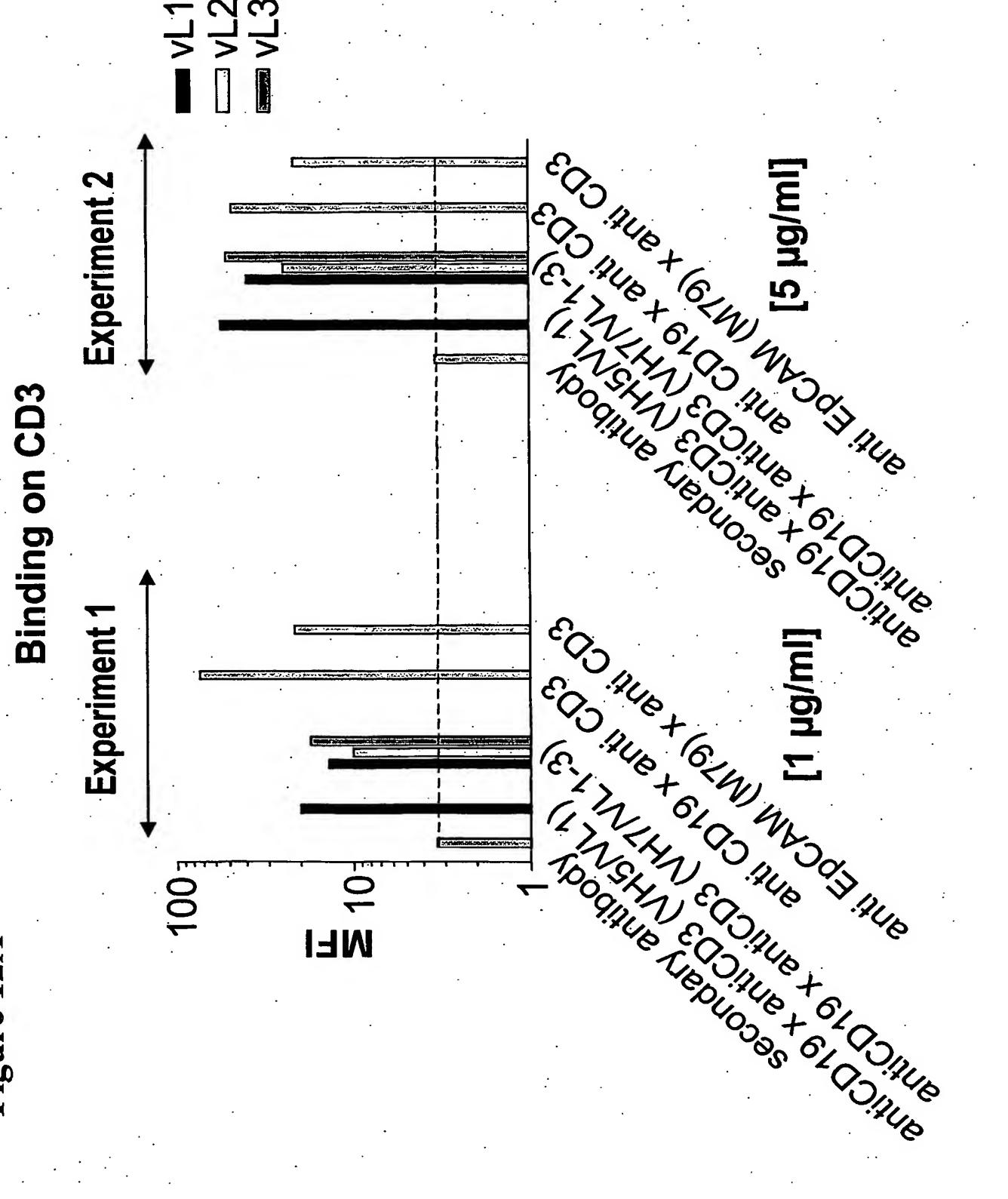
33Kd

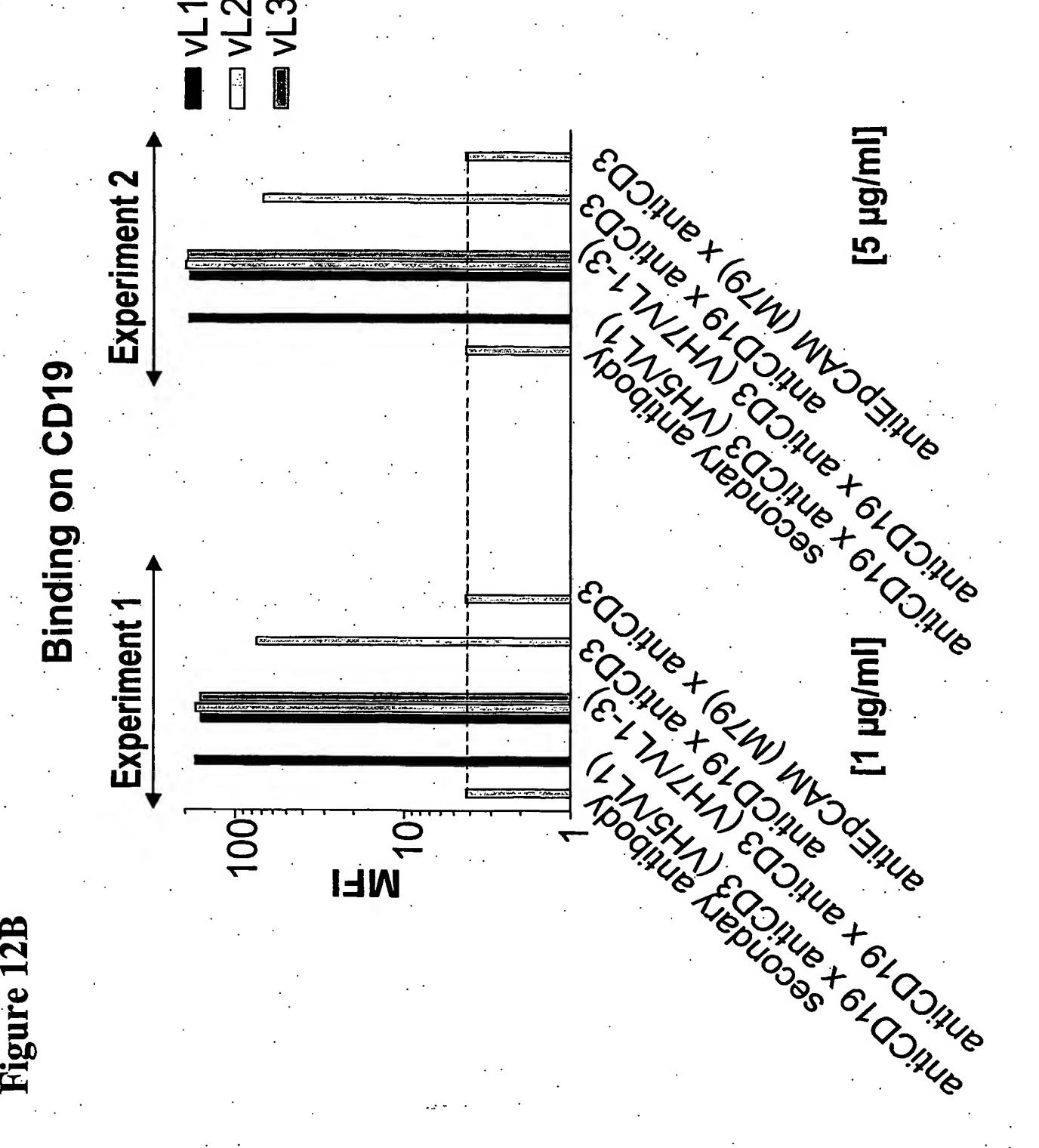


B)

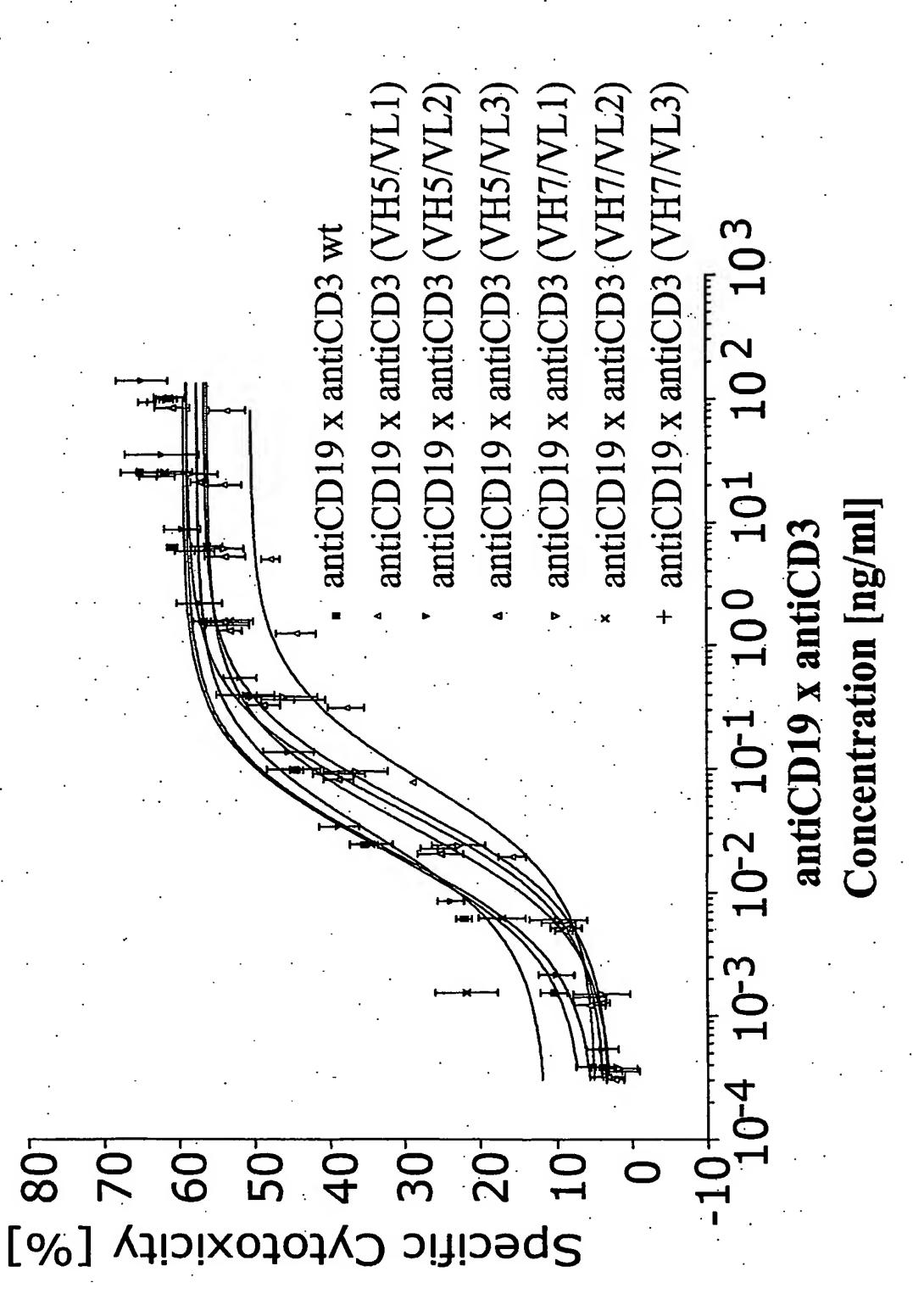










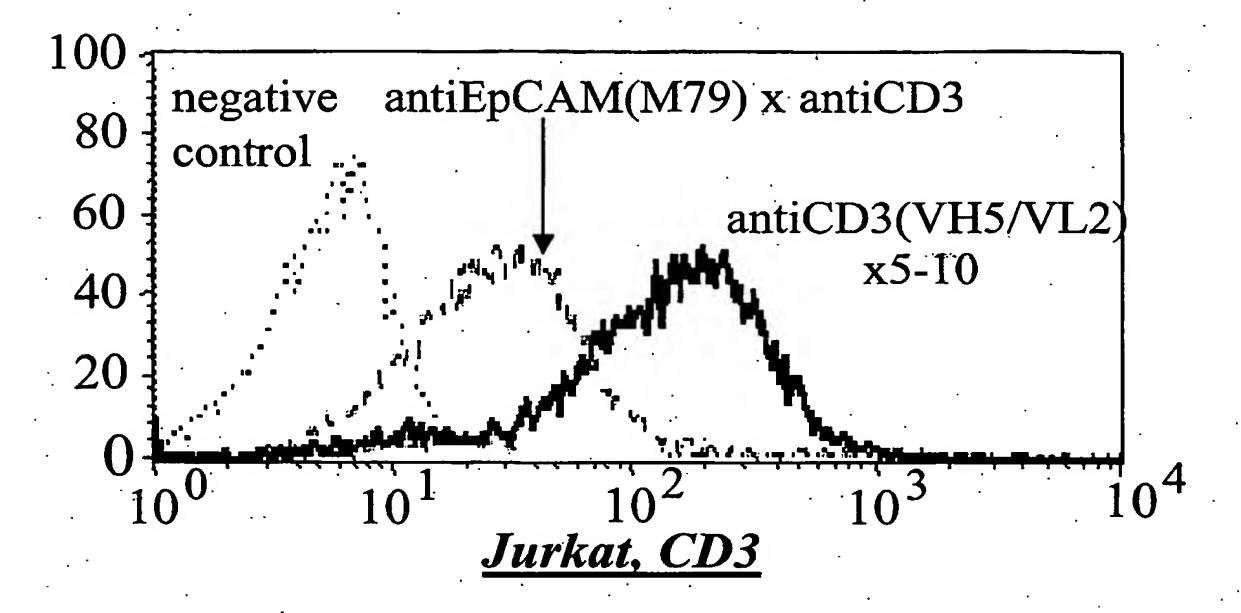


VH3 RVTMTTDTSTSTAYLOMNSLK

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		FRI	CDR1 FI	FR2 CDR2
nondeimmunized				
anti-CD3	DIKLOOSGAELA	RPGASVKMSCKTSGYTET	RYTMHWVKQRPGC	LARPGASVKMSCKTSGYTETRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKD
anti-CD3 VH5	DVQLVQSGAEVKI	KPGASVKVSCKASGYTET	RYTMHWVRQAPGC	VKKPGASVKVSCKASGYTETRYTMHWVRQAPGQGLEWIGYHNPSRGYTNYADSVKG
anti-co3 VH7	DVQLVQSGAEVK	KPGASVKVSCKASGYTET	RYTMHWVRQAPGC	VKKPGASVKVSCKASGYTETRYTMHWVRQAPGQGLEWIGYINPSRGYTNYNQKFKD
anti-CD3 VH2	DVQLVQSGAEVKI	KPGASVKVSCKASGYTAT	RYTMHWVRQAPGC	VKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSRGYTNYAQKLQG
anti-CD3 VH3	DVQLVQSGAEVKI	KPGASVKVSCKASGYTAT	RYTMHWVRQAPGC	VKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYHNPSRGYTNYAQKLQG
	·	FR3	CDR3	FR4
nondeimmunized	'			
anti-CD3	KATLTTDKSSTA	KATLTTDKSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS	RYYDDHYCLDYWG	QGTTLTVSS
anti-co3 VH5		RETITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSS	RYYDDHYCLDYWG	QGTTVTVSS
anti-co3 VH7	RVTLTTDKSTSTAN	RVTLTTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYCLDYWGQGTTVTVSS	RYYDDHYCLDYWG	QGTTVTVSS
anti-co3 VH2	RVTMTTDTSTSTA	anti-cos vaz Rvimitorbistanmelsblrsedtaryycaryyobhycloywgogitvīvss	RYYDDHYCLDYWG	QGTTVTVSS
anti-co3 VH3	RVTMTTDTSTA	anti-cos vas rumatorprestavionneiktedtavyycaryyddhycldymgggttvtvss	RYYDDHYCLDYWG	QGTTVTVSS

Figure 15 A antiCD3(VH5/VL2) x 5-10 (SEQ ID NO: 37)



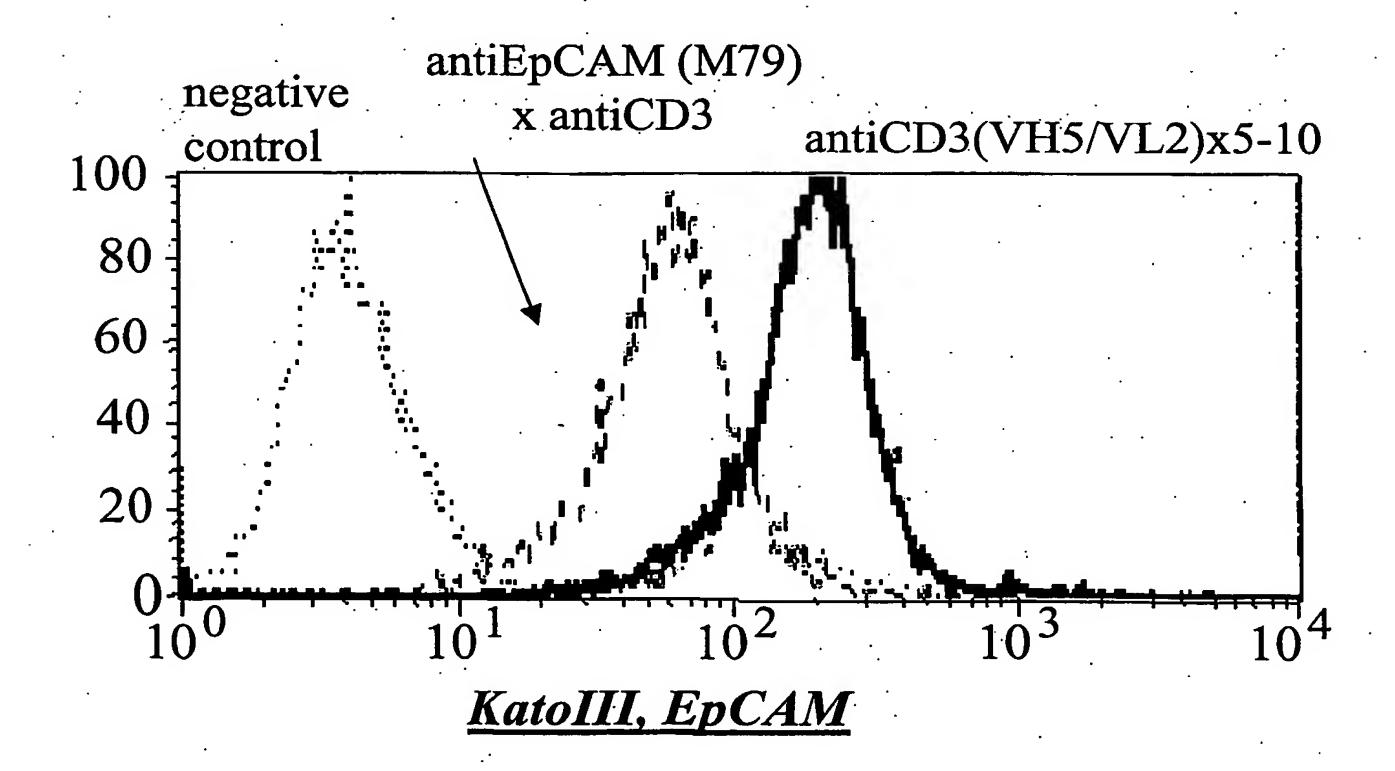
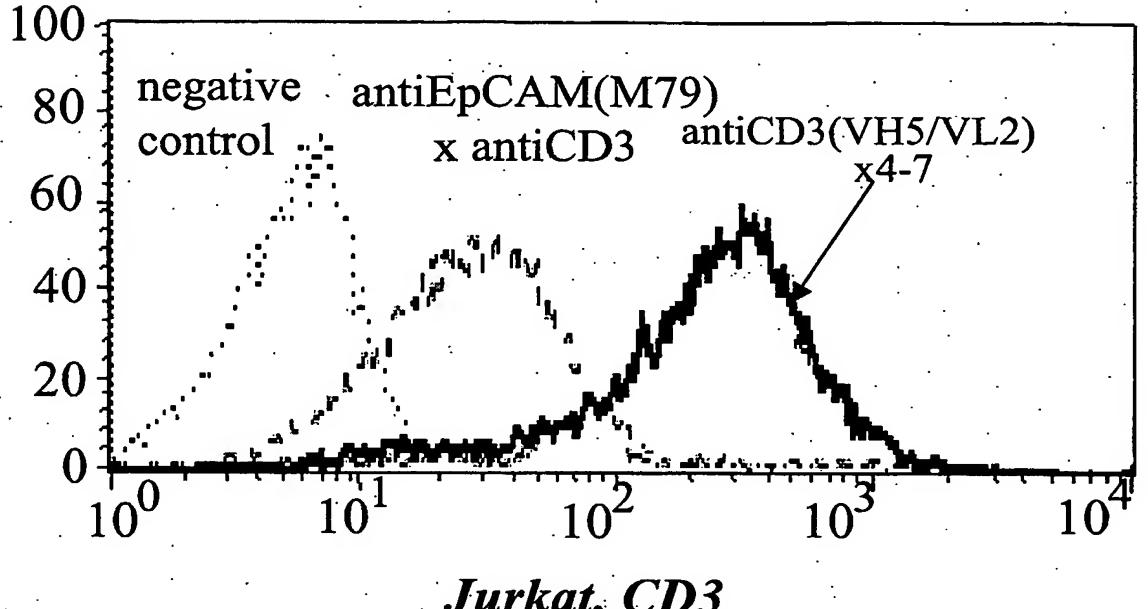


Figure 15B

antiCD3(VH5/VL2) x 4-7 (SEQ ID NO:33)



Jurkat, CD3

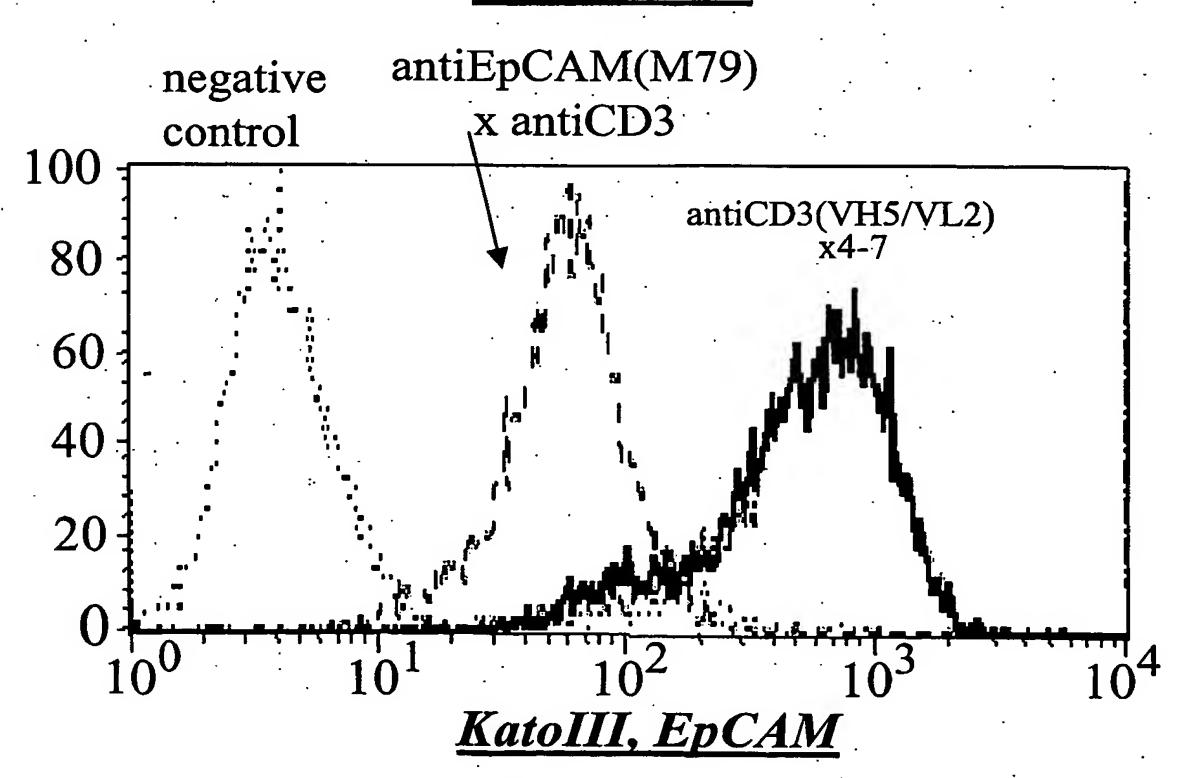
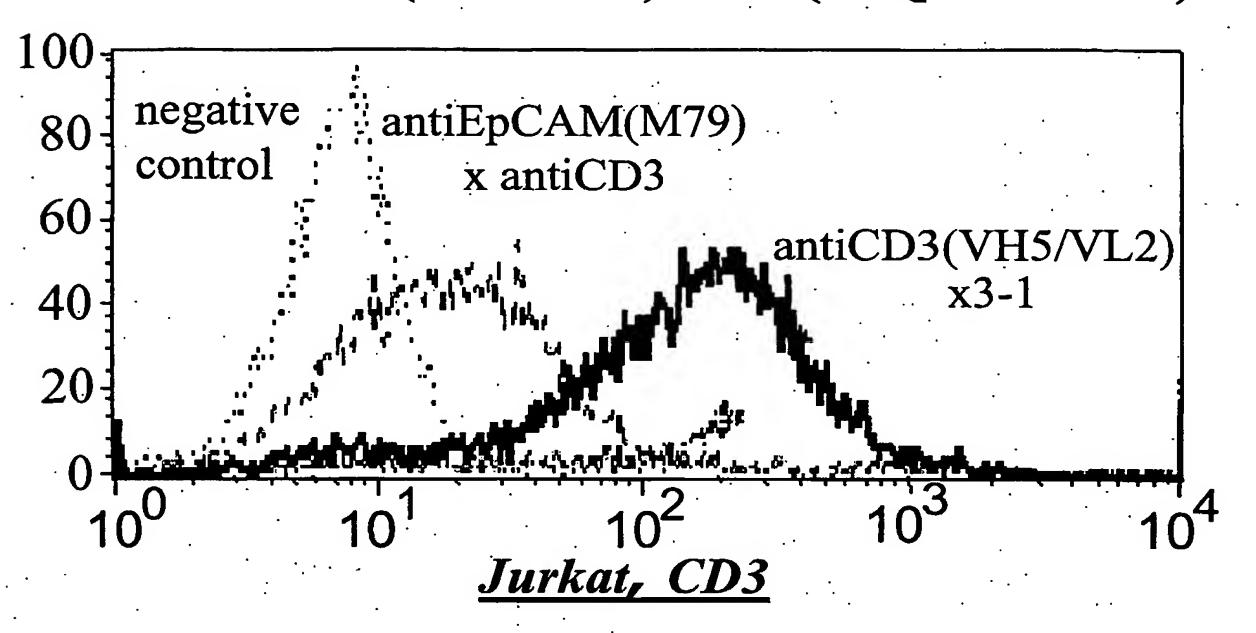


Figure 15C

antiCD3(VH5/VL2) x 3-1 (SEQ ID NO:31)



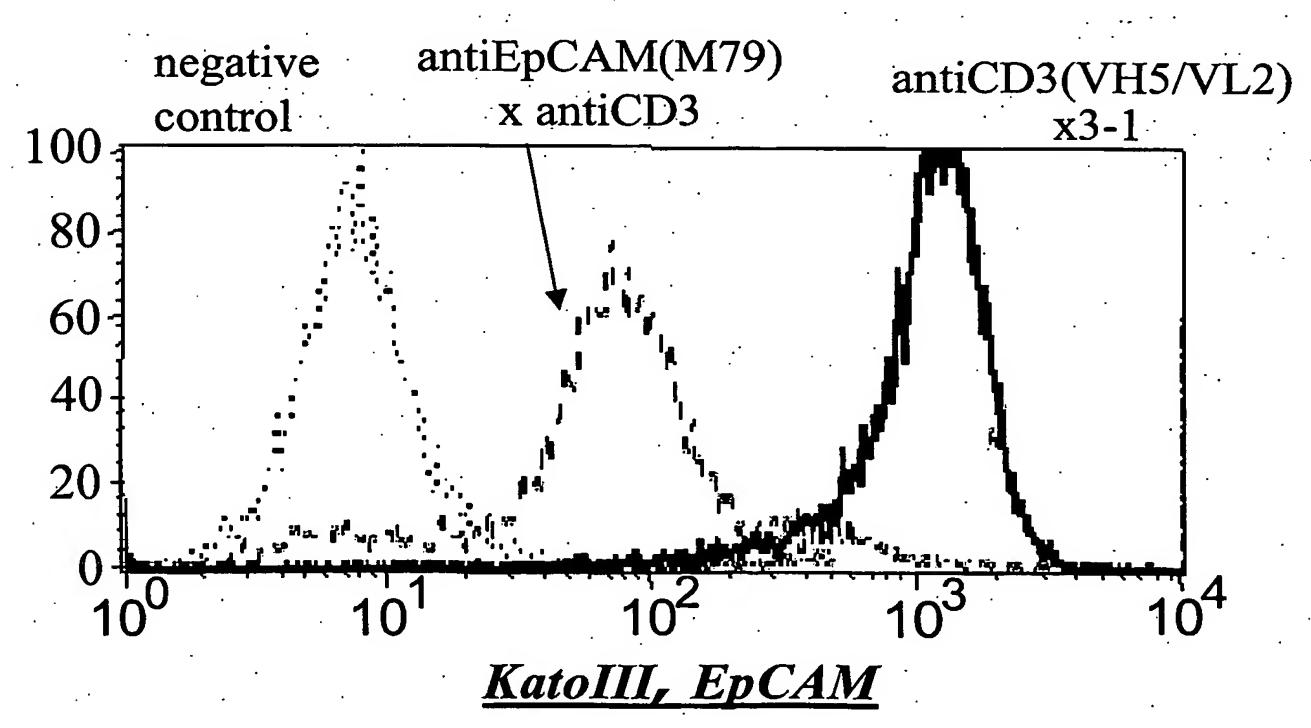
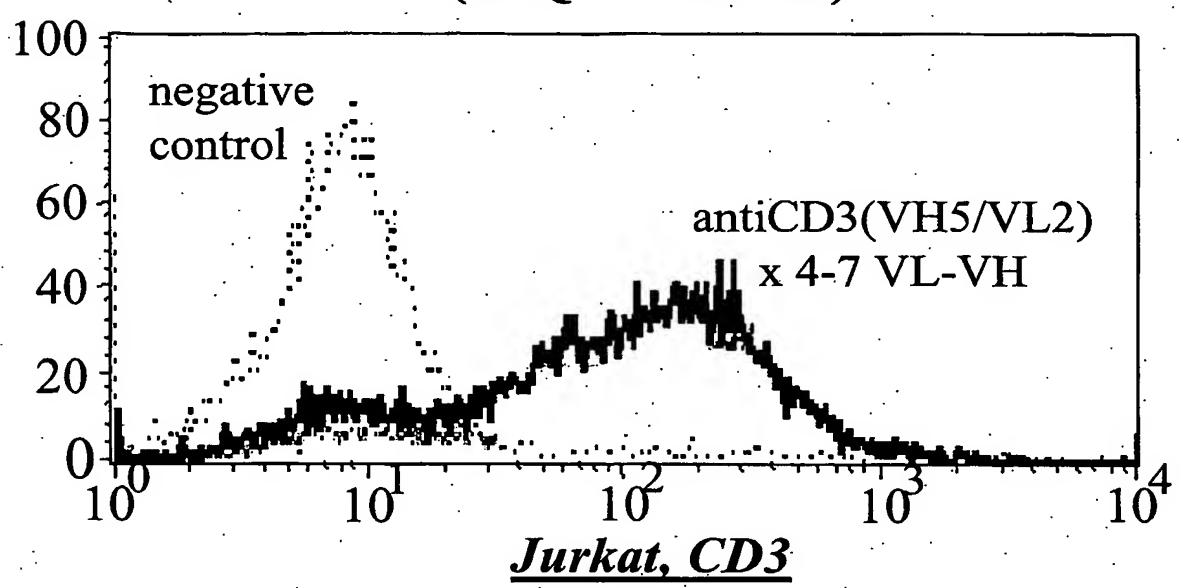
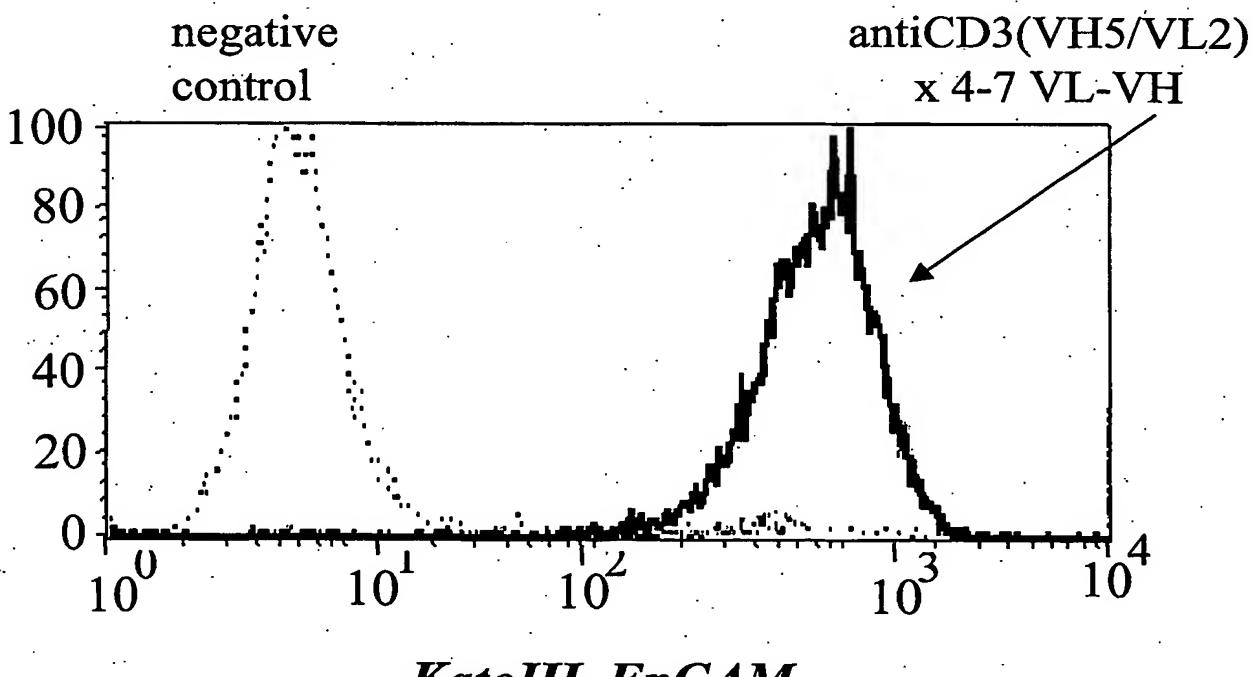


Figure 15 D

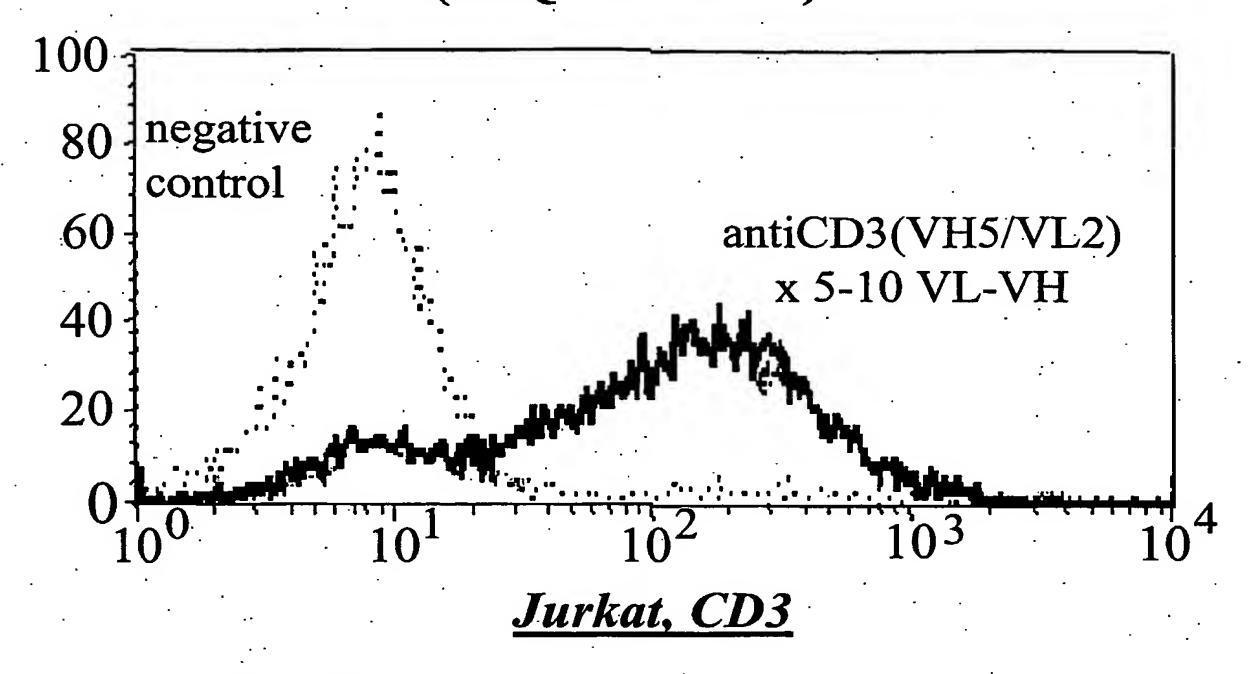
antiCD3(VH5/VL2) x 4-7 VL-VH (SEQ ID NO: 35)





KatoIII, EpCAM

Figure 15 E antiCD3(VH5/VL2) x 5-10 VL-VH (SEQ ID NO:39)



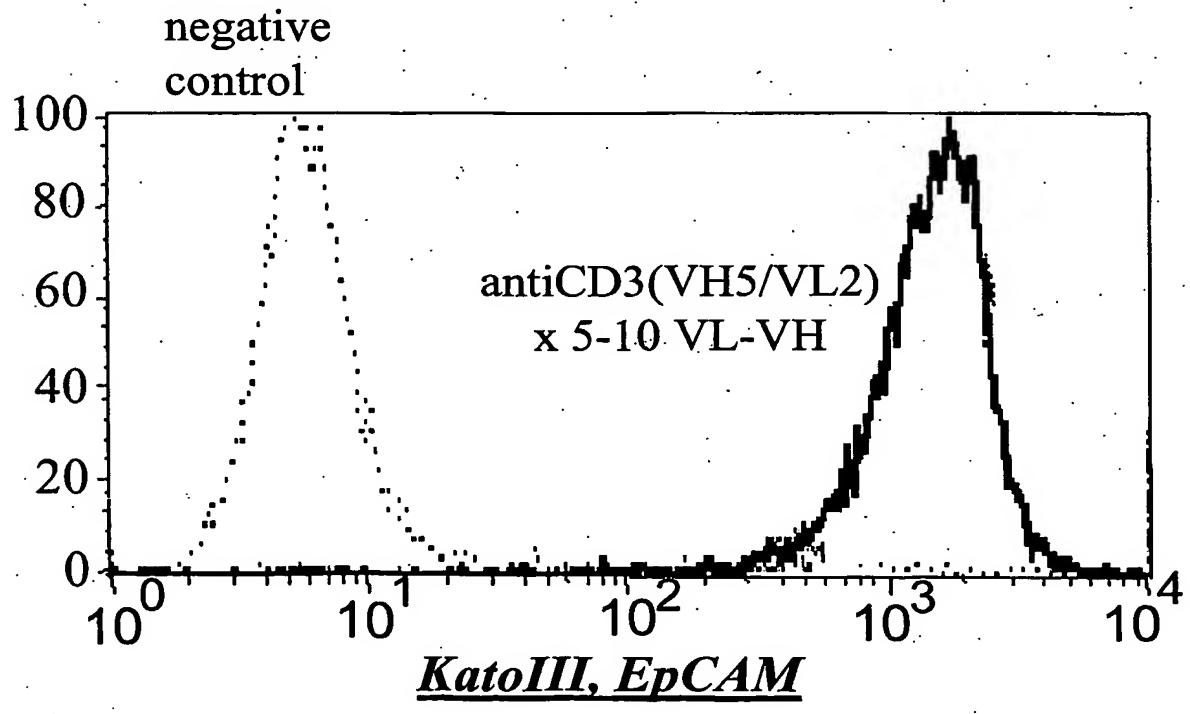


Figure 16

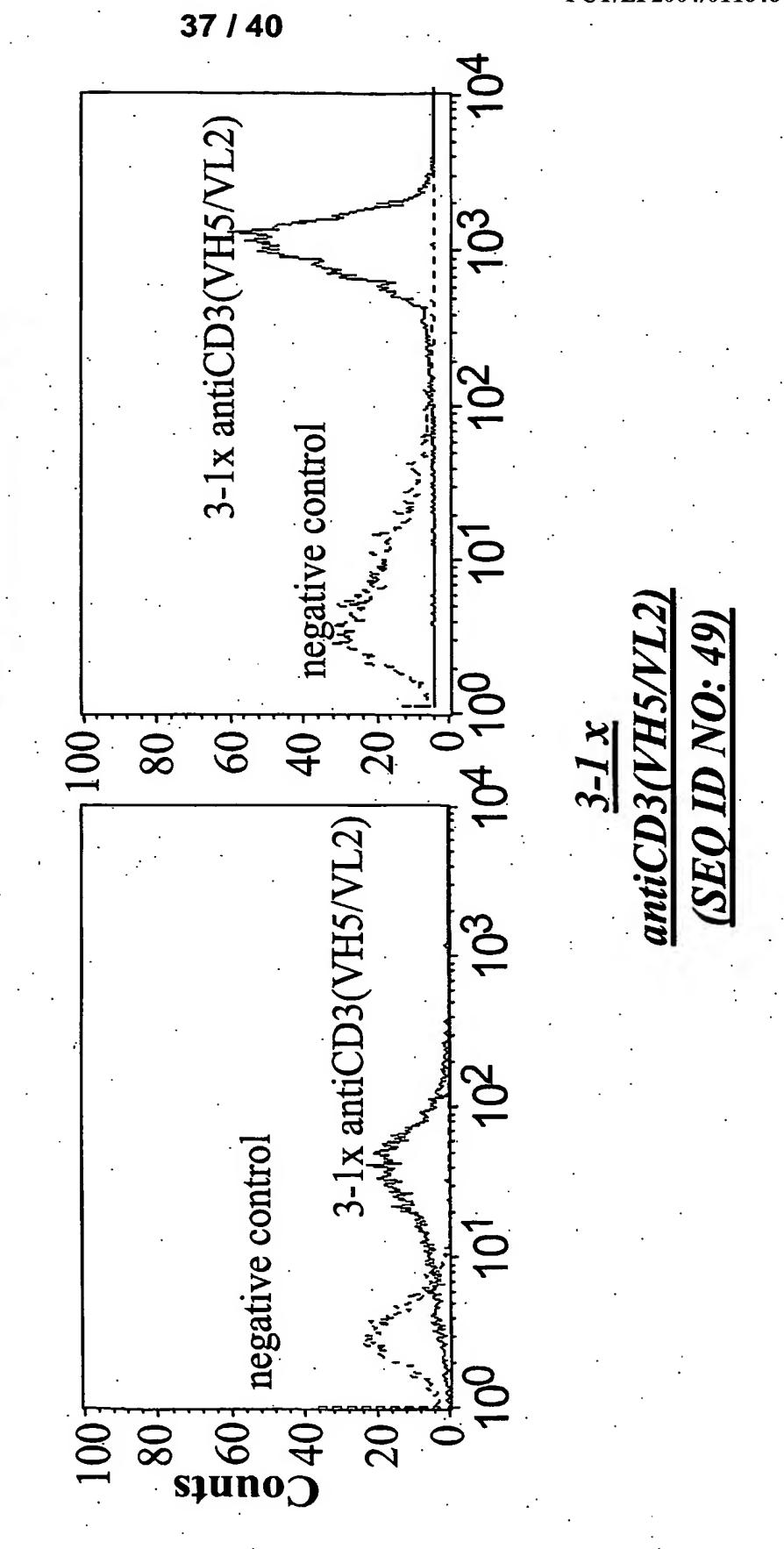


Figure 16

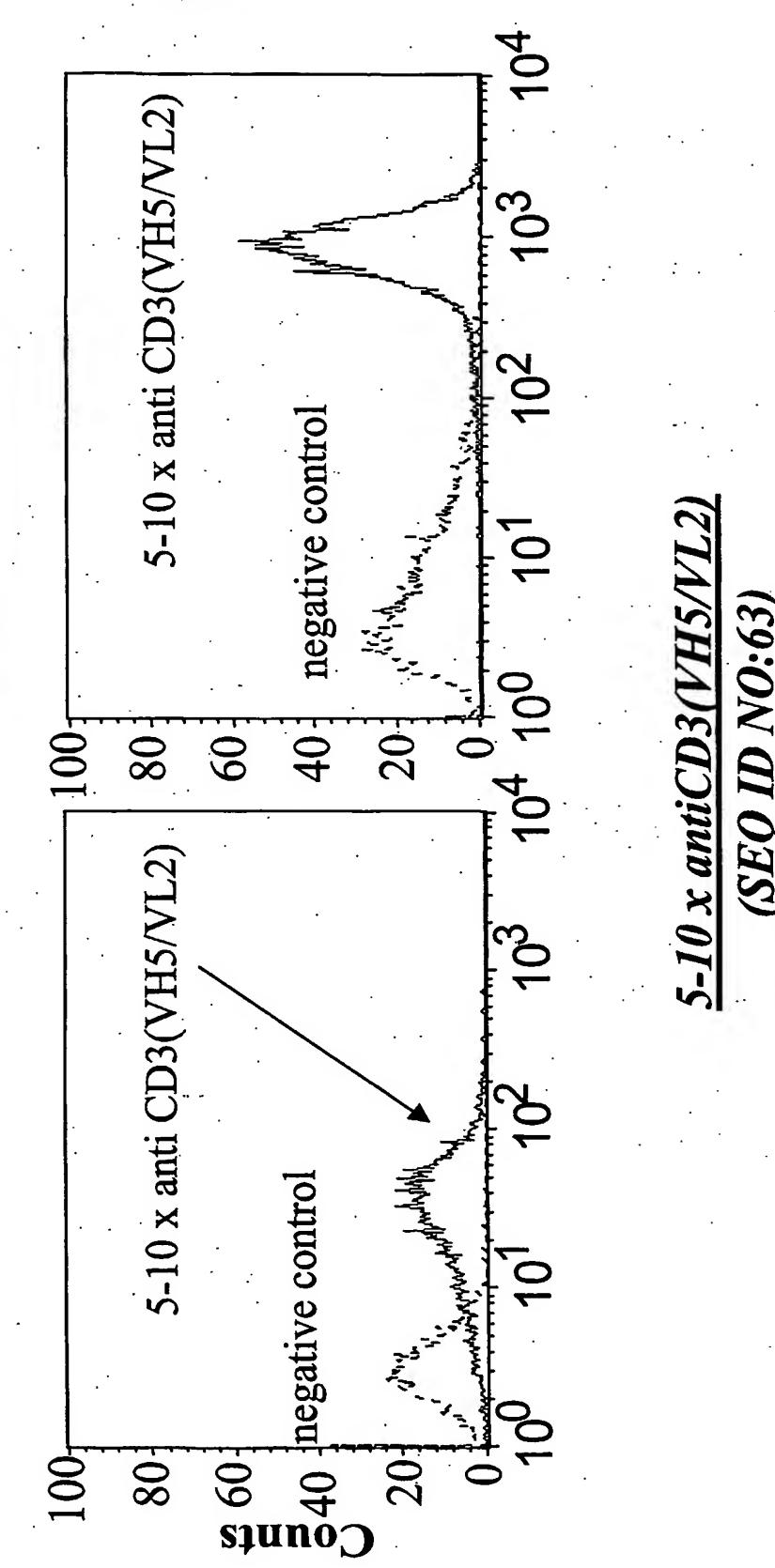
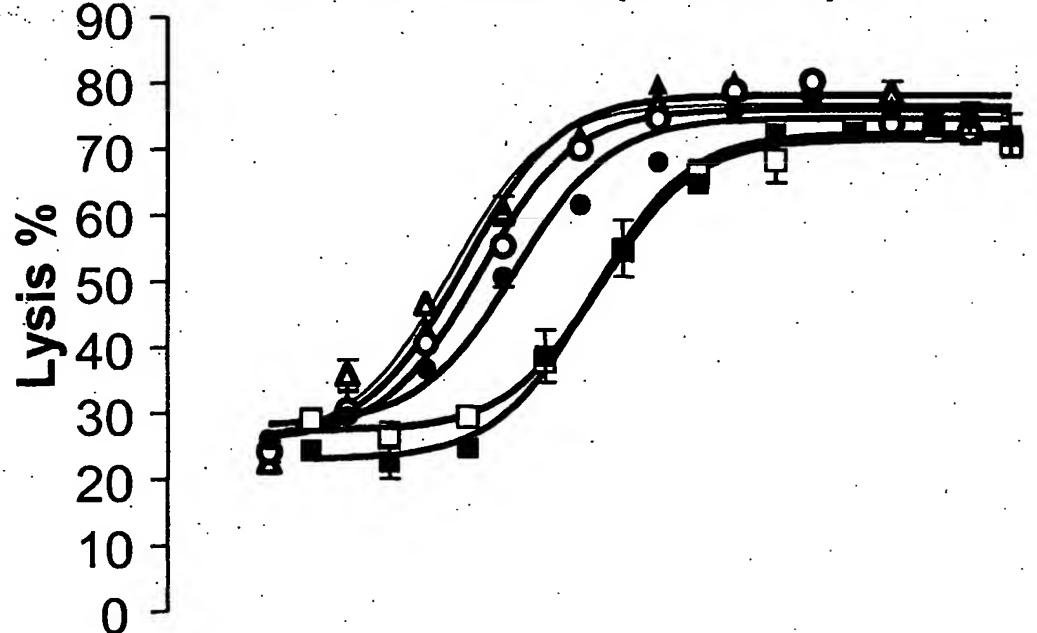


Figure 17

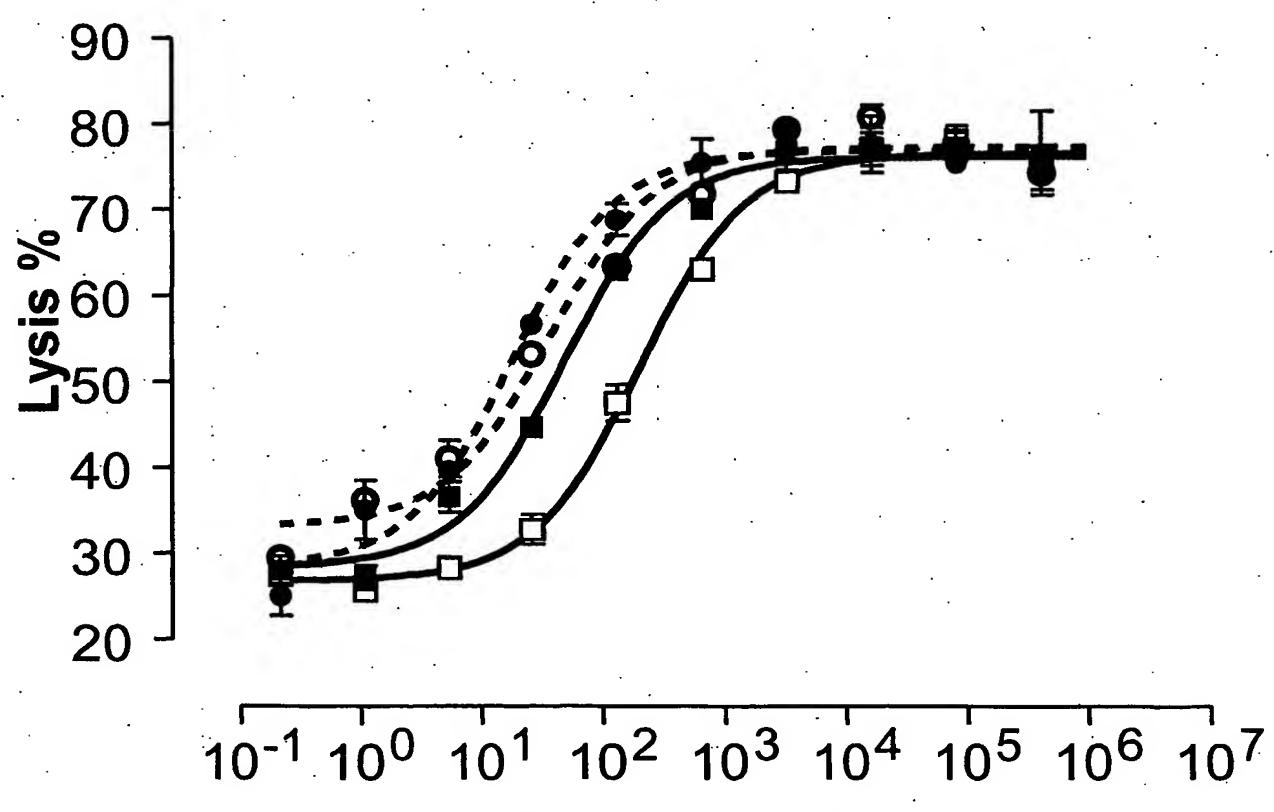
- wt antiCD3 x 3-1
- ☐ di antiCD3(VH5/VL2) x 3-1
- wt antiCD3 x 5-10
- O di antiCD3 (VH5/VL2) x 5-10
- ▲ wt antiCD3 x 4-7
- Δ di antiCD3(VH5/VL2) x 4-7



 $10^{-1}10^{0}10^{1}10^{2}10^{3}10^{4}10^{5}10^{6}10^{7}$ bispecific construct [pg/ml]

Figure 18

- 3-1 x antiCD3
- □ 3-1 x antiCD3(VH5/VL2)
- 5-10 x antiCD3
- o 5-10 x antiCD3(VH5/VL2)



bispecific construct [pg/ml]